Effect of X-Irradiation on the ATP-Inhibition of Phosphorylase *b*

The phosphorylase *b* (E.C. 2.4.1.1.) – one of the key enzymes of the carbohydrate metabolism – is an allosteric enzyme which can exhibit various interactions between sites specific for substrates, and activators or inhibitors respectively[1].

According to our earlier investigations, the allosteric – i.e. regulatory – sites of phosphorylase *b* are far more sensitive to X-rays than is the catalytic centre[2]. These conclusions were drawn from kinetic experiments which indicated the greater radiation sensitivity of the K_m of allosteric activator, AMP, than that of substrate G-1-P.

As is known the substrate saturation curve of phosphorylase *b* has a hyperbolic shape. In the presence of the allosteric inhibitor, ATP, the substrate saturation curve is assumed to have a sigmoid-like shape, proving the cooperative interactions between the allosteric and catalytic sites[1].

In order to confirm our earlier findings, experiments were carried out to study the radiation sensitivity of the above-mentioned cooperative interactions between the different sites.

The preparation and purification procedures of the enzyme were reported elsewhere[2,3]. The activity was determined in the direction of glycogen synthesis. The samples were irradiated in the presence of air at room temperature by a TUR X-ray machine with 2.5 mm Al filter, 200 kV, 15 mA, and the dose rate was 1500 R/min.

Figure 1 shows the substrate saturation curve of phosphorylase *b* in the presence and absence of ATP. Figure 1a shows the saturation curves where the concentration of the activator AMP was 1 m*M*.

It is well observable in Figure 1b that the sigmoid-shape of the saturation curve – in the presence of ATP – can be depressed by decreasing the concentration of AMP up to 0.25 m*M*. At a lower concentration of the activator, the substrate-binding ability of the phosphorylase *b* was weaker, indicated by the more effective inhibition, although the ATP concentration remained unaltered.

Our hypothesis – based on the determination of kinetic contants for AMP and G-1-P, respectively – was that the allosteric cooperative interaction must be damaged to a greater extent by X-rays than the catalytic properties of the enzyme. If the hypothesis was valid one should be able to present the above experimental results not only by altering the AMP concentrations but also by X-irradiation. Figure 2 shows the effect of X-rays on the saturation curves of phosphorylase *b* when it was determined at standard concentrations of AMP and ATP. As it can be seen, 20 and 30 kR depressed the curves particularly at high G-1-P concentrations and the shape of the curves was rather the same as in the case of lower AMP concentrations without irradiation.

These findings are in full agreement with our hypothesis. The X-irradiation affected the cooperative interaction of activator and substrate in the same way as the decreasing of the activator concentration in the presence of standard inhibitor level. The practically unchanged activity at low G-1-P concentrations indicates that the radiation sensitivity of the catalytic centre is really less than that of the AMP-binding capacity, i.e. the sensitivity of allosteric sites. Evidence has been presented that SH-groups may be involved in the binding of AMP but not in the binding of G-1-P[4]. We suggest that these findings may be explained by the greater radiation sensitivity of the regulatory function of phosphorylase *b*, since SH-groups are assumed to be far more radiosensitive than other groups. These model-observations may have biological importance.

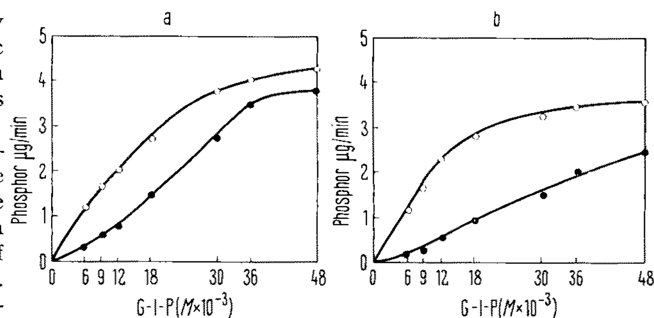

Fig. 1a. The empty circles represent the enzyme activity, expressed in the liberated inorganic phosphor µg/min, plotted in the function of G-1-P concentration. The reaction mixture contained 0.2 ml enzyme solution (10^{-7} *M*) in glycerophosphate buffer, at pH 6.8, and 0.2 ml substrate (G-1-P: 6–48 m*M*; AMP: 1 m*M*; glycogen 1%). The closed circles indicate the effect of 10 m*M* ATP on the enzyme activity.

Fig. 1b. The Figure shows the same experiment as Figure 1a, but the AMP concentration was 0.25 m*M*.

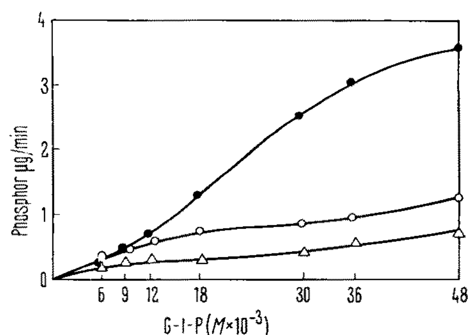

Fig. 2. The closed circles represent the experiment demonstrated in Figure 1a, in the presence of ATP. The effect of 20 kR o——o; and 30 kR △——△, on the sigmoid-like curve of ATP inhibition.

Zusammenfassung. Es wurde die Wirkung der Röntgenbestrahlung auf die ATP-Hemmungskurve der Phosphorylase *b* analysiert und gefunden, dass sich die Sigmoidform der ATP-Hemmungskurve nach der Röntgenbestrahlung vermindert. Die Bestrahlung dürfte die allosterische Eigenschaft von Enzymee beeinträchtigt haben.

S. Damjanovich, J. Sümegi and S. Tóth

Pathophysiological Institute, Medical University School, Debrecen (Hungary),
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